

REMARKS

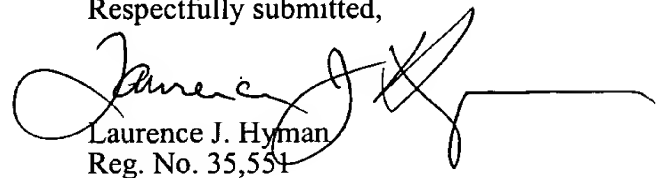
Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-12, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,


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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 20 of page 7 has been amended as follows:

Figure 1: Figure 1 contains the amino acid sequence of SS scFv (SEQ ID NO:1) as deduced from its nucleotide sequence. In the scFv, V_H is connected to V_L by a linker peptide, GVGGSG₄SG₄S (SEQ ID NO:2). The framework regions, CDRs, and linker have been marked. H denotes the heavy chain; L denotes the light chain; FR denotes a framework region; CDR denotes a complementarity determining region.

Paragraph beginning at line 25 of page 7 has been amended as follows:

Figure 2: Mesothelin binding as judged by ELISA of phage clones selected after panning each of the four libraries. Figure 2A: This Figure shows, in separate rows, the amino acid numbering, nucleotide sequence, and amino acid sequence, of the CDR3 of the variable light chain of SS scFv. Top row: Amino acid residue numbers of the variable light chain CDR3 of parental scFv SS. Middle row: Nucleotide sequence (SEQ ID NO:3) encoding the amino acids in the CDR3 of scFv SS CDR3. Rectangles designate the tetranucleotide hotspot motifs; an oval designates the serine hotspot motif in the sequence. Bottom row: Amino acid sequence (SEQ ID NO:4) of the CDR3 of scFv SS, in single letter code.

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Paragraph beginning at line 10 of page 8 has been amended as follows:

Figure 3: Amino acid sequence of some of the ELISA positive phage clones obtained after panning of the different libraries. Only sequences of regions which differ among the clones are shown. An asterisk (*) indicates that several clones with the same amino acid sequence but different nucleotide sequences were found. VL CDR3 nucleotide (SEQ ID NO:3) and amino acid (SEQ ID NO:4) sequences are shown.

Paragraph beginning at line 27 of page 26 has been amended as follows:

While the V_H and V_L regions of some antibody embodiments can be directly joined together, one of skill will appreciate that the regions may be separated by a peptide linker consisting of one or more amino acids. Peptide linkers and their use are well-known in the art. See, e.g., Huston, *et al.*, *Proc. Nat'l Acad. Sci. USA* **8**:5879 (1988); Bird, *et al.*, *Science* **242**:4236 (1988); Glockshuber, *et al.*, *Biochemistry* **29**:1362 (1990); U.S. Patent No. 4,946,778, U.S. Patent No. 5,132,405 and Stemmer, *et al.*, *Biotechniques* **14**:256-265 (1993), all incorporated herein by reference. Generally the peptide linker will have no specific biological activity other than to join the regions or to preserve some minimum distance or other spatial relationship between them. However, the constituent amino acids of the peptide linker may be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity. Single chain Fv (scFv) antibodies optionally include a peptide linker of no more than 50 amino acids, generally no more than 40 amino acids, preferably no more than 30 amino acids, and more preferably no more than 20 amino acids in length. In some embodiments, the peptide linker is a concatamer of the sequence Gly-Gly-Gly-Ser (SEQ ID NO:5), preferably 2, 3, 4, 5, or 6 such sequences. However, it is to be appreciated that some amino acid substitutions within the linker can be made. For example, a valine can be substituted for a glycine.

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Paragraph beginning at line 26 of page 36 has been amended as follows:

In preferred embodiments of the present invention, the toxin is *Pseudomonas* exotoxin (PE). The term "*Pseudomonas* exotoxin" as used herein refers to a full-length native (naturally occurring) PE or a PE that has been modified. Such modifications may include, but are not limited to, elimination of domain Ia, various amino acid deletions in domains Ib, II and III, single amino acid substitutions and the addition of one or more sequences at the carboxyl terminus such as KDEL (SEQ ID NO:6) and REDL (SEQ ID NO:7). See Siegall, *et al.*, *J. Biol. Chem.* **264**:14256-14261 (1989). In a preferred embodiment, the cytotoxic fragment of PE retains at least 50%, preferably 75%, more preferably at least 90%, and most preferably 95% of the cytotoxicity of native PE. In a most preferred embodiment, the cytotoxic fragment is more toxic than native PE.

Paragraph beginning at line 5 of page 38 has been amended as follows:

As noted above, some or all of domain 1b may be deleted, and the remaining portions joined by a linker or directly by a peptide bond. Some of the amino portion of domain II may be deleted. And, the C-terminal end may contain the native sequence of residues 609-613 (REDLK; SEQ ID NO:8), or may contain a variation found to maintain the ability of the construct to translocate into the cytosol, such as REDL (SEQ ID NO:7) or KDEL (SEQ ID NO:6), and repeats of these sequences. See, e.g., U.S. Patents 5,854,044; 5,821,238; and 5,602,095 and WO 99/51643. While in preferred embodiments, the PE is PE4E, PE40, or PE38, any form of PE in which non-specific cytotoxicity has been eliminated or reduced to levels in which significant toxicity to non-targeted cells does not occur can be used in the immunotoxins of the present invention so long as it remains capable of translocation and EF-2 ribosylation in a targeted cell.

Paragraph beginning at line 20 of page 52 has been amended as follows:

Phagemid pPSC 7-1 is a phage antibody display vector coding for the mesothelin binding SS(scFv) (Chowdhury1998). Analysis of the nucleotide sequence of SS(scFv) revealed 32 hot-spots. Of these 32 hotspots three were selected for targeting mutations. These are located in the CDR3 of the VL encoding residues 89, 90, 92, 93 and 94. Single stranded uracil containing DNA of pPSC 7-1 was prepared as described earlier (Chowdhury *et al.* J. Mol. Biol. 281:917-928 (1998)). A stop codon and a diagnostic HpaI restriction site was introduced into the CDR3 of the VL by Kunkel's mutagenesis (Kunkel, T.A., Proc. Natl. Acad. Sci. USA 82:488-492 (1985)) to produce the phagemid pPSC 7-1-94. The stop codon was introduced to prevent over representation of the wild-type sequence in the library since this mutagenesis approach gives a background of 10-12%. Uracil containing ssDNA of pPSC 7-1-94 was used as a template to construct two experimental and two control libraries using degenerate oligos. Oligo SS VL 89/93/94 5'GCACCGAACGTGAGAGG SNNSNNACTCCACTGSNNGCAGTAATAAGTTGC 3' (SEQ ID NO:9) was -used for making the library Lib 89/93/94. This oligo randomizes codons 89, 93 and 94 of the VL in SS(scFv) with all twenty different amino acids. As with all the other libraries described below, this oligo replaces the stop codon at position 94 with that of a tyrosine and does not create ocher and opal stop codons.

Paragraph beginning at line 38 of page 52 has been amended as follows:

The second library, Lib 92-94 was made using oligo SS VL Mut 92-94, 5' GCACCGAACGTGAG AGGSNNSNNSNCCACTGCTGGCAGTAATAAG 3' (SEQ ID NO:10) which randomizes residues 92-94. Residues 92-94 are encoded by two different hotspots placed side by side. The third and fourth libraries were made as controls. The third library, Lib 89-91, was made with the oligo SS VL Mut 89-91, 5'GCACCGAACGTGAGAGGGTAACCACTSNNSNNS NNGCAGTAATAAGTTGC

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(SEQ ID NO:11) 3' which randomizes residues 89-91. Residue 90 is a conserved residue in a hotspot. Residue 91 falls outside of the hotspot. The fourth library Lib 95-97 was made with the oligo SS VL Mut 95-97 5'CTTTGTCCCAGCACC GAASNNSNNSNNGTAACCACTCCACTGCTGCG 3' (SEQ ID NO:12). It randomizes residues 95-97 all of which fall outside the hotspots.